The Circular Dichroism of Polypeptide Films*

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ABSTRACT: Circular dichroism spectra were obtained on films of polypeptides in the β conformation. In agreement with previous optical rotatory dispersion results, these polypeptides fall into two categories: I-β and II-β. Poly-L-tryptophan was also examined in film form, identifying the side-chain bands above 250 mμ.

In recent years, a major effort has been devoted to the characterization of polypeptide and protein structures by the method of optical rotatory dispersion (Urnes and Doty, 1961; Fasman, 1963; Harrington *et al.*, 1966). The recent availability of instrumentation capable of measuring circular dichroism in the far-ultraviolet region has made it possible to identify the circularly dichroic bands which give rise to the Cotton effects in the optical rotatory dispersion spectra of various conformations. As a result, solutions of polypeptides in various conformations have been examined and the proper circular dichroism bands identified. Thus, it is known that, in solution, the α-helical conformation is characterized by a positive band at 190–191 mμ and two negative bands at 207 and 221 mμ, while the random (or disordered) conformation has a strong negative band at 196 mμ, a weak positive band at 217 mμ, and a very weak negative band at 238 mμ (Holzwarth *et al.*, 1962; Brahms and Spach, 1963; Grosjean and Tari, 1964; Beychok and Fasman, 1964; Holzwarth and Doty, 1965; Velluz and Legrand, 1965; Townend *et al.*, 1966; Sarkar and Doty, 1966; Timasheff *et al.*, 1967). Polyproline I and II, as well as the collagen triple helix, have been similarly characterized (Timasheff *et al.*, 1967). The β or pleated-sheet structure received attention after the discovery that poly-L-lysine will assume that conformation in dilute aqueous solution when heated gently at alkaline pH values (Rosenheck and Doty, 1961; Applequist and Doty, 1962; Davidson *et al.*, 1966). It was found that the antiparallel pleated-sheet β conformation (Susi *et al.*, 1967) assumed by that polypeptide in solution has a circular dichroism spectrum consisting of a negative band at 217 mμ (Townend *et al.*, 1966; Sarkar and Doty, 1966) and a positive band at 195 mμ (Townend *et al.*, 1966). The latter may split into two components (Timasheff *et al.*, 1967) as predicted by theory (Pysh, 1966).

Recently, Fasman and Potter (1967) reported that films of polypeptides, known from infrared dichroism to be in the antiparallel β structure, give nonidentical optical rotatory dispersion spectra which may be grouped into two families, designated as I-β and II-β. The first has an optical rotatory dispersion spectrum similar to that of β-structured poly-L-lysine in solution (trough at 230 mμ, peak at 205 mμ) (Davidson *et al.*, 1966). The second family (II-β) has an optical rotatory dispersion spectrum composed of a trough close to 240 mμ and a peak between 210 and 215 mμ. The latter optical rotatory dispersion spectrum is similar to that of β-structured poly-*S*-carboxymethyl-L-cysteine in aqueous solution at pH values below 5 (Ikeda and Fasman, 1967). It seemed of interest, therefore, to examine the circular dichroic spectra of β-structured polypeptides cast as films and to establish the band shifts which occur between forms I and II.

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TABLE I: Circular Dichroism Band Positions of β-Structure-Forming Polypeptides.

Polypeptide	Band Position (mμ)
Poly-L-Lysine	
β conformation	219 (−); 207 (c-o);[a] + below 207
α helix	221 (−); 209 (−); 201 (c-o); 191.5 (+)
Random	202 (−); 220–230 (−) sh
Poly-*O*-acetyl-L-serine	216 (−); 203 (c-o); 196 (+) (optical rotatory dispersion similar to Fasman and Potter, 1967).
Poly-*O*-*t*-butoxy-L-serine	220 (−); 212 (c-o); 200 (+)
Polyvaline	217 (−); 203 (c-o); 198 (+) (optical rotatory dispersion similar to Fasman and Potter, 1967).
Poly-*S*-carbobenzoxymethyl-L-cysteine	228 (−); 217 (c-o); 203 (+) (optical rotatory dispersion similar to Fasman and Potter, 1967).
Poly-*S*-carboxymethyl-L-cysteine (solution, pH 4.42)	227 (−); 213 (c-o); 198 (+)

[a] Abbreviation used: c-o, crossover point.

Materials and Methods

The poly-α-amino acids used were identical with those previously employed in the optical rotatory dispersion studies (Fasman *et al.*, 1965; Fasman and Potter, 1967; Ikeda and Fasman, 1967). The β-conformation-forming polymers were dissolved in trifluoroacetic acid and films were cast by evaporation of solutions layered on quartz disks. Each disk with the film on it was carefully mounted as a window on a demountable sample cell (from which the second window had been removed) for alignment in the instrument. All the sample preparation work was performed in a glove bag, containing potassium hydroxide, under a mild stream of dry nitrogen. The films were finally dried in a vacuum desiccator before use. The circular dichroism spectra were obtained with a Durrum-Jasco ORD/UV 5 apparatus.[1]

Results and Discussion

β Structures. The results obtained with a number of films are summarized in Table I and typical circular dichroism spectra are shown on Figure 1. It was found that the circular dichroism spectra of the β-structured polypeptides fall into the same two categories as the optical rotatory dispersion patterns. In the first, I-β, the spectrum displays a positive band between 196 and 200 mμ and a negative band between 216 and 220 mμ, similar to the previous observations on β-structured poly-L-lysine in solution. In the second case, II-β, the circular dichroism spectrum, is shifted to higher wavelengths, just as in the optical rotatory dispersion. The circular dichroism shift is particularly pronounced in the high-wavelength negative band (~228 mμ).

In order to ascertain the effect that drying may have on the circular dichroism spectra of polypeptides, poly-L-lysine films were cast from solutions in which this poly-α-amino acid assumes α-helical, antiparallel β, and random conformations. The results are shown on Figure 2. In the first two cases, the films were cast from aqueous pH 11.0 solutions at room temperature, where the conformation is that of an α helix. The spectrum of the dried film was measured and found to have negative bands at 221 and 209 mμ and a positive band at 191.5 mμ, *i.e.*, in excellent agreement with positions established for that conformation in solution. This film was then heated for 10 min at 52°. The resulting spectrum displayed a negative band at 219 mμ, a crossover point at 207 mμ, and was rising toward a positive maximum at 195 mμ. Thus, in the α-helical and β structures, casting of the polypeptide as a film neither affects the circular dichroism spectrum nor greatly hinders the α–β transformation.

On the other hand, a film cast at pH 7.5, where the polypeptide is in random conformation, gave a spectrum with a negative band at 202 mμ and shallow broad negative absorption between 220 and 230 mμ. This spectrum is quite different from the one observed in solution at the same pH. The result is not surprising, however, since in a dried film the polypeptide chain is not capable of undergoing the random flight structural fluctuations characteristic of a random coil in solution. In the film, definite constraints are imposed on the chain, and as a result, the structure, while probably displaying no periodicity, is no longer that of a random coil in constant thermal motion. It is particularly interesting that this spectrum displays the same characteristics as those observed in the circular dichroism spectra of some unordered and denatured proteins (Timasheff *et*

[1] Mention of companies or products is for the convenience of the reader and does not constitute an endorsement by the U. S. Department of Agriculture.

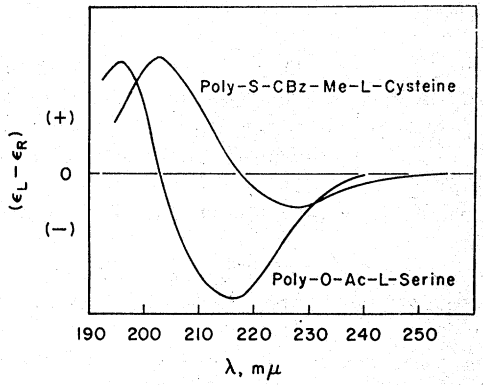

FIGURE 1: Circular dichroism spectra of poly-*S*-carbobenzoxymethyl-L-cysteine and poly-*O*-acetyl-L-serine films.

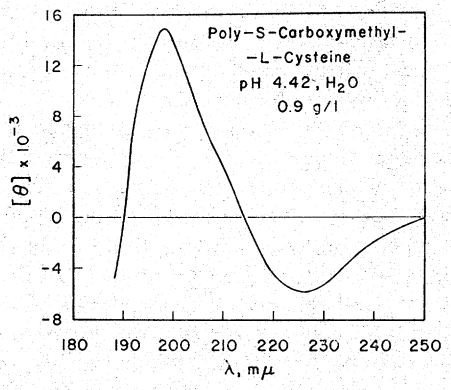

FIGURE 3: Circular dichroism spectrum of β-structured poly-*S*-carboxymethyl-L-cysteine in solution.

al., 1967; Townend *et al.*, 1967; Timasheff and Stevens, 1968); these show a negative circular dichroism band close to 205 mμ and have a shallow negative absorption in the region of 220–230 mμ. It may be expected that in the unordered portions of the three-dimensional structure of a protein, the conformation is not truly that of a random coil, but is highly constrained by side-chain interactions and disulfide bridges. The same constraints exist in unordered proteins, such as α_s-casein, and proteins denatured without breaking S–S bonds (*e.g.*, by alkaline denaturation). It might seem that the circular dichroism spectrum of unordered poly-L-lysine cast as a film should be a better model for the spectrum of the unordered portions of a protein than a randomly coiled polypeptide in aqueous solution.

Having established that the I-β poly-α-amino acids belong to a class typified by poly-L-lysine in solution, it was interesting to see whether the parallel between the II-β films and poly-*S*-carboxymethyl-L-cysteine in solution observed in optical rotatory dispersion is maintained in circular dichroism. For this purpose the circular dichroism spectrum of the latter polypeptide was obtained in aqueous solution at pH 4.42 and is shown in Figure 3. It is evident that the II-β character of poly-*S*-carboxymethyl-L-cysteine is maintained also in circular dichroism. The spectrum has a positive band at 198 mμ, a negative one at 227 mμ, and a crossover point at 214 mμ. The molar ellipticities, $[\theta]$, of the peaks are $+14.9 \times 10^3$ and -5.9×10^3 (deg cm³)/dmole, respectively. The position and intensity of the negative band are in excellent agreement with the values reported by Ikeda and Fasman (1967). It would appear, thus, that the classification of β-structured polypeptides into I-β and II-β is maintained in the circular dichroism spectra as well as in optical rotatory dispersion both in solution and in films. Optical rotatory dispersion spectra measured on all the investigated films were essentially identical with those of Fasman and Potter (1967). It is possible, in this way, to establish the dichroic bands which give rise to the two types of optical rotatory dispersion spectra found for antiparallel β structures. The nature of interactions causing the shift in the high-wavelength ($n-\pi^-$) band is not known. It has been shown, however, that β-structured polypeptides are strongly affected by stabilizing side-chain interactions (Davidson and Fasman, 1967; Birshtein and Ptitsyn, 1967); in all probability, the observed shifts reflect such interactions, as well as steric effects due to side-chain bulkiness, which may cause small variations in the orientation of the transitions moments of the β-structure backbone chain. It should be noted, furthermore, that, while the band positions of the two types of structures are essentially the same for various polypeptides, the relative intensities

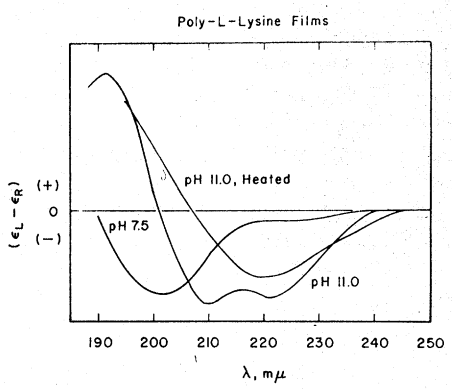

FIGURE 2: Circular dichroism spectra of poly-L-lysine films in the α-helical, antiparallel β, and random conformations.

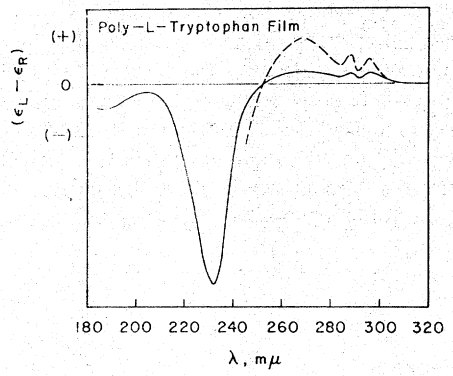

FIGURE 4: Circular dichroism spectrum of poly-L-tryptophan film. The dashed line represents the spectrum obtained above 250 mμ on a film of greater thickness.

of the bands depend upon the side chains. This may be related to differences in polarity of the peptide-bond environment (Iizuka and Yang, 1966) caused by various side-chain conformations.

Poly-L-tryptophan. Another spectral region of great interest in the optical rotatory dispersion and circular dichroism of proteins is the near-ultraviolet region (250–300 mμ), in which aromatic chromophoric side chains undergo optically active transitions. The circular dichroism spectrum of poly-L-tyrosine has been examined by Beychok and Fasman (1964). A similar examination of poly-L-tryptophan was impeded by the fact that this polymer is not soluble in any solvents which are transparent to ultraviolet light. Fasman *et al.* (1965), however, have examined the optical rotatory dispersion of this polypeptide in the form of films cast from dimethylformamide solution. In this study it was shown that poly-L-tryptophan exists in the form of an α helix. Poly-L-tryptophan films were cast, therefore, and examined by circular dichroism between 185 and 320 mμ. A typical spectrum is shown on Figure 4. In the near-ultraviolet region this spectrum is characterized by three positive bands at 296, 288, and 269 mμ; the last band is very broad and probably represents more than one transition. These bands account for the complex positive Cotton effects observed in the same spectral region by Fasman *et al.* (1965) in optical rotatory dispersion experiments. Below 250 mμ, the circular dichroism spectrum becomes negative with a deep band at 232 mμ, a negative minimum at 205 mμ, and a weak positive extremum at 188 mμ. It is not possible to interpret the spectrum in this region in terms of any known polypeptide conformation. Quite obviously the observed spectrum represents a complicated superposition between the α-helical bands which must be present (Fasman *et al.*, 1965) and strong aromatic chromophore bands which are present in this wavelength region. Rotation in the instrument of partially oriented films results in complex changes in the spectrum below 250 mμ, with positive and negative extrema appearing and disappearing as a function of the angle of rotation. Above 250 mμ, rotation of the film has little effect on the qualitative features of the spectrum. The band intensities change with angle; however, both band dichroic positions and sign remain unchanged. The identification of the three positive bands above 250 mμ should be of help in assigning spectral features of proteins. For example, β-lactoglobulin has negative bands at 293 and 285 mμ, which are apparently unaffected by acetylation of the tyrosines (Timasheff *et al.*, 1967; Townend *et al.*, 1968). This would support their assignment to tryptophan transitions, a conclusion in good agreement with the results on poly-L-tryptophan reported in this communication.

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